

Glomerular Hyperfiltration and Renal Disease Progression in Type 2 Diabetes

PIERO RUGGENENTI, MD^{1,2}
 ESTEBAN L. PORRINI, MD¹
 FLAVIO GASPARI, CHEMD¹
 NICOLA MOTTERLINI, STATSCID¹
 ANTONIO CANNATA, CHEMIST¹
 FABIOLA CARRARA, CHEMIST¹
 CLAUDIA CELLA, PHARMCHEMD¹
 SILVIA FERRARI, CHEMIST¹
 NADIA STUCCHI, CHEMIST¹

ANELIYA PARVANOV, MD¹
 ILIAN ILIEV, MD¹
 ALESSANDRO ROBERTO DODESINI, MD³
 ROBERTO TREVISAN, MD³
 ANTONIO BOSSI, MD⁴
 JELKA ZALETEL, MD⁵
 GIUSEPPE REMUZZI, MD^{1,2}
 FOR THE GFR STUDY INVESTIGATORS*

OBJECTIVE—To describe the prevalence and determinants of hyperfiltration (glomerular filtration rate [GFR] ≥ 120 mL/min/1.73 m²), GFR decline, and nephropathy onset or progression in type 2 diabetic patients with normo- or microalbuminuria.

RESEARCH DESIGN AND METHODS—We longitudinally studied 600 hypertensive type 2 diabetic patients with albuminuria <200 μ g/min and who were retrieved from two randomized trials testing the renal effect of trandolapril and delapril. Target blood pressure (BP) was $<120/80$ mmHg, and HbA_{1c} was $<7\%$. GFR, albuminuria, and glucose disposal rate (GDR) were centrally measured by iothexol plasma clearance, nephelometry in three consecutive overnight urine collections, and hyperinsulinemic euglycemic clamp, respectively.

RESULTS—Over a median (range) follow-up of 4.0 (1.7–8.1) years, GFR declined by 3.37 (5.71–1.31) mL/min/1.73 m² per year. GFR change was bimodal over time: a larger reduction at 6 months significantly predicted slower subsequent decline (coefficient: -0.0054 ; SE: 0.0009), particularly among hyperfiltering patients. A total of 90 subjects (15%) were hyperfiltering at inclusion, and 11 of 47 (23.4%) patients with persistent hyperfiltration progressed to micro- or macroalbuminuria versus 53 (10.6%) of the 502 who had their hyperfiltration ameliorated at 6 months or were nonhyperfiltering since inclusion (hazard ratio 2.16 [95% CI 1.13–4.14]). Amelioration of hyperfiltration was independent of baseline characteristics or ACE inhibition. It was significantly associated with improved BP and metabolic control, amelioration of GDR, and slower long-term GFR decline on follow-up.

CONCLUSIONS—Despite intensified treatment, patients with type 2 diabetes have a fast GFR decline. Hyperfiltration affects a subgroup of patients and may contribute to renal function loss and nephropathy onset or progression. Whether amelioration of hyperfiltration is renoprotective is worth investigating.

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Early glomerular filtration rate (GFR) elevation plays a central role in the pathogenesis and progression of renal disease in experimental diabetes (1). Small cohort studies suggest that type 1

and type 2 diabetic subjects with glomerular hyperfiltration may be at increased risk of accelerated renal function loss or progression to micro- or macroalbuminuria (2–7), but findings were not confirmed

in other series (8,9). These inconsistencies are most likely explained by small sample size and heterogeneity of the above studies that enhanced random data fluctuations. Moreover, no study evaluated the interactions between treatment effects on glomerular hyperfiltration and subsequent disease progression.

To address whether and to what extent glomerular hyperfiltration predicts faster GFR decline or increased risk of onset or progression of nephropathy and whether amelioration of hyperfiltration may be renoprotective in the long-term, we took advantage of a homogeneous cohort of 600 type 2 diabetic patients with normo- or microalbuminuria included in the Bergamo Nephrologic Diabetes Complications Trial-B (BENEDICT-B, NCT00235014 at <http://clinicaltrials.gov>) (10) and the Delapril and Manidipine for Nephroprotection in Diabetic nephropathy (DEMAND, NCT00157586 at <http://clinicaltrials.gov>) study (11) who had their GFR prospectively monitored by iothexol plasma clearance (12), a gold standard procedure for GFR determination (13,14). We first described the GFR and its changes over time and then addressed the relationships between potential risk factors for progressive renal dysfunction, including initial hyperfiltration and subsequent GFR decline or new onset of micro- or macroalbuminuria in this population.

RESEARCH DESIGN AND METHODS

This cohort study included subjects from two randomized, double-blind, placebo-controlled clinical trials, BENEDICT-B (10) and DEMAND (11), designed to evaluate the effect of ACE inhibitor therapy on onset and progression of nephropathy in hypertensive type 2 diabetic patients with normo- or microalbuminuria. The two study populations were considered together since they were selected, monitored, and treated according to similar predefined guidelines (see detailed Research Design and Methods in the Supplementary Data online). In all patients, the GFR was centrally measured at the laboratories of the Clinical Research Center of the Mario Negri Institute for Pharmacological Research with

From the ¹Clinical Research Center for Rare Diseases Aldo & Cele Daccò, Mario Negri Institute for Pharmacological Research, Bergamo, Italy; the ²Unit of Nephrology, Azienda Ospedaliera Ospedali Riuniti di Bergamo, Bergamo, Italy; the ³Unit of Diabetology, Azienda Ospedaliera Ospedali Riuniti di Bergamo, Bergamo, Italy; the ⁴Unit of Diabetology, Treviglio Hospital, Treviglio, Italy; and the ⁵Department of Endocrinology, Diabetes and Metabolic Diseases, University Medical Center, Ljubljana, Slovenia.

Corresponding author: Piero Ruggenenti, pruggenenti@ospedaleiruniti.bergamo.it.

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the iohexol (Omnipaque 300; GE Healthcare, Milan, Italy) plasma clearance technique (13,15) at baseline and every 6 months thereafter.

Glucose disposal rate (GDR) was assessed at inclusion and at 1 year in a subgroup by the hyperinsulinemic euglycemic clamp (16). Albumin was measured by nephelometry (Beckman Array System) in three timed overnight urine collections, and HbA_{1c} was measured by ion-exchange high-performance liquid chromatography (normal range: 3.53–5.21%). Other parameters were evaluated with a Beckman Synchron CX5 instrument and a Coulter MaxM (Beckman Coulter).

Definitions

Patients who had a measured GFR at inclusion exceeding the upper limit of the normal range (120 mL/min/1.73 m²) were a priori categorized as “hyperfiltering,” and those with lower GFRs were categorized as “nonhyperfiltering.” Since the reproducibility range of GFR measurement by the iohexol plasma clearance technique is $\pm 6.28\%$ (13,15), a predefined cutoff of 10% GFR reduction largely exceeding the reproducibility range of the measurement was expected to univocally identify patients with true GFR reductions from those with random data fluctuations related to the variability of the method. Thus, among patients who were hyperfiltering at baseline, those with a GFR reduction $>10\%$ at month 6 were considered as patients with ameliorated hyperfiltration. Those with smaller reductions were categorized as “persistently hyperfiltering.”

Predefined end points were 1) the rate of GFR decline over time (GFR slope) defined as the regression line between repeated GFR measurements and time (17,18) and 2) time to onset of persistent micro- or macroalbuminuria defined as urinary albumin excretion (UAE) ≥ 20 and <200 $\mu\text{g}/\text{min}$ or ≥ 200 $\mu\text{g}/\text{min}$, respectively (10,11).

Statistical analysis

Analyses were performed at the Laboratory of Biostatistics of the Clinical Research Center by SPSS 14.0.1 (Chicago, IL), STATA 11.0, and SAS 9.1 (SAS Institute, Inc., Cary, NC). Data were expressed as mean \pm SD, median and interquartile range (IQR), or number and percent as appropriate. $P < 0.05$ indicated statistical significance.

Baseline and follow-up characteristics were compared by paired or unpaired

t test, Wilcoxon rank sum test, χ^2 test, or Fisher exact test. Correlations between variables were evaluated using Pearson r or Spearman ρ correlation coefficients.

GFR slope analyses were preplanned for a subgroup of patients from the BENEDICT study (19) and for all patients from the DEMAND study (11) who had at least three follow-up GFR measurements in addition to baseline GFR. GFR changes over time were a priori evaluated by a single-slope linear model (14) and by a two-phase model in which GFR changes from baseline to month 6 and GFR slope from month 6 to study end were assessed separately (6,17,20). To test the possibility that GFR reduction at 6 months could be affected by GFR at baseline (regression to the mean), GFR changes at 6 months were a posteriori compared between hyperfiltering and nonhyperfiltering patients after adjusting for baseline GFR values by using an ANCOVA (21).

The relationships between baseline GFR, or GFR changes from baseline to month 6, and subsequent GFR decline were evaluated by two multivariable models considering as outcomes the GFR slopes calculated throughout the whole follow-up period (model 1) or from month 6 to study end (model 2), respectively. To account for possible heterogeneity between studies, we developed a meta-analysis of individual patient continuous outcome data using a random trial effect with patient-level covariates in which the original study and the observation from an individual patient were at the highest and lowest level, respectively (22). The possibility of including treatments as a random instead of a fixed effect was eventually assessed by the likelihood ratio test. In additional sensitivity analyses, we further evaluated the bimodal GFR change over time by considering repeated GFR measures as the outcome in a spline function with a knot at 6 months (23). In subjects with at least one follow-up measurement of albuminuria, we a priori evaluated the association between baseline GFR, or hyperfiltration at inclusion, and the development of micro- or macroalbuminuria by Kaplan-Meier analysis and log-rank test and by a multivariate proportional hazards model considering time to micro- or macroalbuminuria as the dependent variable. In addition, we included in the model potential confounders with a proven or expected relationship with the outcome, such as age, sex, baseline albuminuria, baseline HbA_{1c}, and systolic or diastolic BP, randomization to an ACE

inhibitor yes or no, and inclusion in the DEMAND or BENEDICT-B trial. The same approach was used in post hoc analyses evaluating progression to micro- or macroalbuminuria in patients with hyperfiltration at inclusion who were persistently hyperfiltering at 6 months, as compared with patients who had their hyperfiltration at inclusion ameliorated at 6 months considered alone or in combination with those who were persistently nonhyperfiltering since inclusion. The assumption of proportionality was assessed by the log-minus-log procedure.

RESULTS—A total of 4,593 GFRs were measured over a median (range) follow-up of 4.0 (1.75–8.11) years. Baseline measurements were available in 600 subjects, including all 377 patients from DEMAND and 223 of the 281 (78.4%) subjects from BENEDICT-B (Supplementary Fig. 1). The last visit was in July 2008.

Baseline characteristics

Of the patients, 90 (15%) had a GFR ≥ 120 mL/min/1.73 m² at inclusion. Compared with the 510 with lower GFRs, these 90 patients were younger; had higher HbA_{1c}, blood glucose, and serum triglyceride levels; and had lower serum creatinine, uric acid levels, GDR, blood pressure (BP), and albuminuria but similar BMI (Table 1). The proportions of patients who were microalbuminuric or were eventually randomized to ACE inhibitor therapy were similar between groups. GFR was similar in patients with normo- or microalbuminuria or with or without ACE inhibitor therapy (Supplementary Fig. 2A).

Outcomes

GFR decline. Of the 600 included patients, 449 (74.8%) had at least four GFR measurements, including baseline (median [IQR]: 9 [8–11]), available for GFR slope analyses. Of the 151 patients with fewer GFR measurements, 61 and 42 withdrew consent to trial participation or to GFR measurement, respectively. Thus, in only 48 (8%) case subjects were GFR measurements incomplete because of clinical reasons, including death, adverse events, progression to macroalbuminuria, and other reasons (Supplementary Fig. 1). Characteristics of patients with or without GFR slope data were similar (Supplementary Table 1). Throughout the whole study period,

Table 1—Baseline characteristics of all study patients considered as a whole, with those who were hyperfiltering or nonhyperfiltering at inclusion considered separately

Characteristic	Overall	Hyperfiltering	Nonhyperfiltering
Number of patients	600	90	510
Demographic/clinical			
Age (years)	61.3 ± 7.8	57.3 ± 7.0	61.9 ± 7.8*
Known duration of diabetes (years)	7 (3–13)	6 (3–14)	7 (3–13)
BMI (kg/m ²)	29.3 ± 4.4	30.1 ± 4.6	29.2 ± 4.4
Smoking status, n (%)			
Never	272 (45.3)	36 (40.0)	236 (46.3)
Former	240 (40.0)	38 (42.2)	202 (39.6)
Current	88 (14.7)	16 (17.8)	72 (14.1)
Trough BP (mmHg)			
Systolic	149.8 ± 15.2	150.1 ± 14.2	149.7 ± 15.4
Diastolic	87.8 ± 9.3	88.3 ± 8.0	87.7 ± 9.6
Laboratory			
HbA _{1c} (%) ^F	6.2 ± 1.6	6.7 ± 1.6	6.1 ± 1.6*
Glucose (mg/dL)	170.6 ± 49.1	190.2 ± 51.1	167.1 ± 47.9*
Triglycerides (mg/dL) [†]	123.5 (90.0–173.7)	142.0 (99.5–241.4)	121.0 (88.0–168.3)*
Cholesterol (mg/dL)**			
Total	199.1 ± 35.7	200.9 ± 41.0	198.8 ± 34.7
LDL	153.1 ± 34.5	156.8 ± 37.9	152.4 ± 33.9
HDL	45.6 ± 11.8	44.1 ± 13.2	45.9 ± 11.5
Uric acid (mg/dL)	5.3 ± 1.3	4.8 ± 0.9	5.5 ± 1.3*
Serum creatinine (mg/dL) [¶]	0.9 ± 0.2	0.8 ± 0.1	0.9 ± 0.1*
GFR (mL/min/1.73 m ²)	101.0 ± 19.6	132.2 ± 11.5	95.5 ± 11.1
UAE (μg/min)	9.9 (4.2–31.6)	9.2 (4.8–38.5)	9.9 (4.1–29.8)
Microalbuminuria, n (%)	210 (35.0)	33 (36.7)	177 (34.7)
GDR (mg/kg/min) ^φ	5.6 ± 2.6	4.9 ± 2.5	5.8 ± 2.7*
Therapy			
Randomization to ACE inhibition, n (%)	436 (72.7)	69 (76.7)	367 (72.2)
Antihypertensive drugs, n			
Baseline	2 (1–3)	2 (1–3)	2 (1–3)
Follow-up	2 (1–3)	3 (2–4)	3 (2–4)
Antidiabetic treatments (%)			
Baseline (Diet/Oral/Oral + Ins/Ins)	19.9/59.6/10.6/9.9	13.3/68.9/13.3/4.4	18.8/62.1/10.0/9.0
Follow-up (Diet/Oral/Oral + Ins/Ins)	25.0/45.3/16.1/13.7	17.8/47.8/20.0/14.4	26.3/44.3/15.5/13.9

Data are mean ± SD or median (IQR). * $P < 0.05$ vs. hyperfiltering. ^FHbA_{1c} was measured by ion-exchange high-performance liquid chromatography (normal range, 3.5–5.2%). To convert percent HbA_{1c} values to International Federation of Clinical Laboratory Medicine (IFCC) units (mmol/mol), use the formula (present HbA_{1c} – 0.956) × 11.145. [†]To convert values for triglycerides to millimoles per liter, multiply by 0.01129. ^{**}To convert values for cholesterol to millimoles per liter, multiply by 0.02586. [¶]To convert values for serum creatinine to micromoles per liter, multiply by 88.4 ^φMeasured in 219 subjects (178 nonhyperfiltering and 41 hyperfiltering).

the GFR declined by 3.37 (5.26–1.64) mL/min/1.73 m² per year. The decline was similar between patients with normo- or microalbuminuria as well as between those randomized to ACE inhibitor or non-ACE inhibitor therapy, whereas it was faster in subjects who were hyperfiltering at inclusion (Table 2). Faster decline was largely explained by a 13-fold larger GFR reduction at month 6 in hyperfiltering subjects ($P < 0.0001$ vs. nonhyperfiltering), whereas the subsequent rate of GFR decline was similar between groups (Table 2 and Supplementary Fig. 2B–D). Larger short-term GFR reduction in hyperfiltering subjects was unlikely to be explained by regression toward the mean

since GFR reduction from baseline to month 6 was significantly different between hyperfiltering and nonhyperfiltering subjects also after adjusting for baseline GFR ($P = 0.018$). Long-term GFR decline was similar in both groups (3.24 [5.5–1.0] vs. 3.09 [5.4–0.7] mL/min/1.73 m² per year, $P = 0.94$) even when GFR values at 6 months were not considered. Short- and long-term GFR changes were similar in micro- and normoalbuminuric patients and in patients with or without ACE inhibitor therapy (Table 2 and Supplementary Fig. 2B–D).

GFR decline tended to be faster in subjects with persistent hyperfiltration than in those who had their hyperfiltration

at inclusion ameliorated at 6 months or who were nonhyperfiltering since inclusion (4.19 [7.7–1.6] vs. 3.23 [5.25–1.26] mL/min/1.73 m² per year, $P = 0.09$). The difference achieved statistical significance in sensitivity analyses considering the rate of GFR decline in patients with persistent hyperfiltration as compared with that observed in those who had their hyperfiltration at inclusion ameliorated at 6 months (4.19 [11.3–1.6] vs. 1.72 [7.0 to –0.5] mL/min/1.73 m² per year, $P = 0.01$) (Fig. 1D). Percent reduction in mean BP (3.15 [10.8 to –1.4] vs. 10.06% [15.5 to –2.4], $P = 0.041$), blood glucose (0.03 [17.0–23.8] vs. 11.26% [25.8 to –0.2], $P = 0.030$), and GDR

Table 2—GFR changes during the study

	GFR decline from baseline	GFR reduction at month 6	GFR decline from month 6
Overall	3.37 (5.26–1.64)	3.04 ± 15.22	3.37 (5.57–1.31)
Hyperfiltering	4.39 (6.87–2.58)	13.64 ± 19.04	2.49 (5.53–1.30)
Nonhyperfiltering	3.23 (5.12–1.38)	1.18 ± 13.65	3.38 (5.57–1.32)
P value	0.0017	<0.0001	0.737
Microalbuminuria	2.95 (4.89–1.51)	4.27 ± 13.40	2.66 (5.03–1.16)
Normoalbuminuria	3.58 (5.31–1.68)	2.48 ± 15.96	3.50 (5.69–1.42)
P value	0.117	0.220	0.139
ACE inhibitor therapy			
Yes	3.35 (5.24–1.77)	3.16 ± 15.14	3.31 (5.33–1.31)
No	3.38 (5.36–1.47)	2.71 ± 15.50	3.53 (5.77–1.44)
P value	0.895	0.785	0.663

Data are medians (IQR) or means ± SD. All data were normally distributed and show the rate of GFR decline from baseline to study end (mL/min/1.72 m² per year), GFR changes from baseline to month 6 (mL/min/1.72 m²), and GFR decline from month 6 to study end (mL/min/1.72 m² per year).

(13.09 [28.4 to –1.06] vs. –7.74% [8.4 to –17.3], $P = 0.033$) at month 6 versus baseline was significantly smaller in patients with persistent hyperfiltration than in those who had their hyperfiltration at inclusion ameliorated at month 6 (Fig. 1A–C). The above differences were not explained by baseline characteristics, the proportion of patients allocated to ACE inhibitor therapy, and the distribution of antihypertensive and antidiabetic drugs at baseline and on follow-up that were all similar between groups (Supplementary Table 2). Data were not fully explained

by suboptimal metabolic or BP control since in the group with persistent hyperfiltration, blood glucose and HbA_{1c} were <125 mg/dL and <6.5% in 24 and 46.5% of patients and systolic and diastolic BP were <130 and <80 mmHg in 24.3 and 38% of case subjects, respectively.

Patients with short-term GFR reduction >10% at month 6 compared with patients with smaller reductions had slower GFR decline on subsequent follow-up in the study group considered as a whole (2.05 [4.47–0.50] vs. 3.90 [5.83–1.56] mL/min/1.73 m² per year,

$P < 0.0001$) as well as in the subgroup of patients without glomerular hyperfiltration at inclusion (2.20 [4.49–0.44] vs. 3.74 [5.78–1.56] mL/min/1.73 m² per year, $P < 0.0001$). Independent of GFR changes from baseline to month 6, patients with hyperfiltration (GFR >120 mL/min/1.73 m²) or without hyperfiltration at month 6 showed a similar GFR loss on follow-up (4.01 [6.6–0.6] vs. 2.26 [5.0–1.3] mL/min/1.73 m² per year, respectively, $P = 0.30$).

Predictors of GFR decline. Results of univariable analyses are shown in Supplementary Table 3. At multivariable analysis, higher GFR at inclusion (coefficient [SE]: –0.006 [0.0007]; $P < 0.0001$), in addition to older age and higher systolic BP, predicted a faster GFR decline throughout the whole study period (Supplementary Table 4, model 1). The predictive value of baseline GFR was lost (–0.0016 [0.0008]; $P = 0.050$) when GFR decline from month 6 to study end was considered as an outcome variable (Supplementary Table 4, model 2). When GFR change from baseline to month 6 was considered in the model instead of baseline GFR, the GFR change was the strongest predictor of subsequent slope (–0.0054 [0.0009]; $P < 0.0001$), and a larger reduction from baseline to month 6 was significantly associated with a slower slope on subsequent follow-up

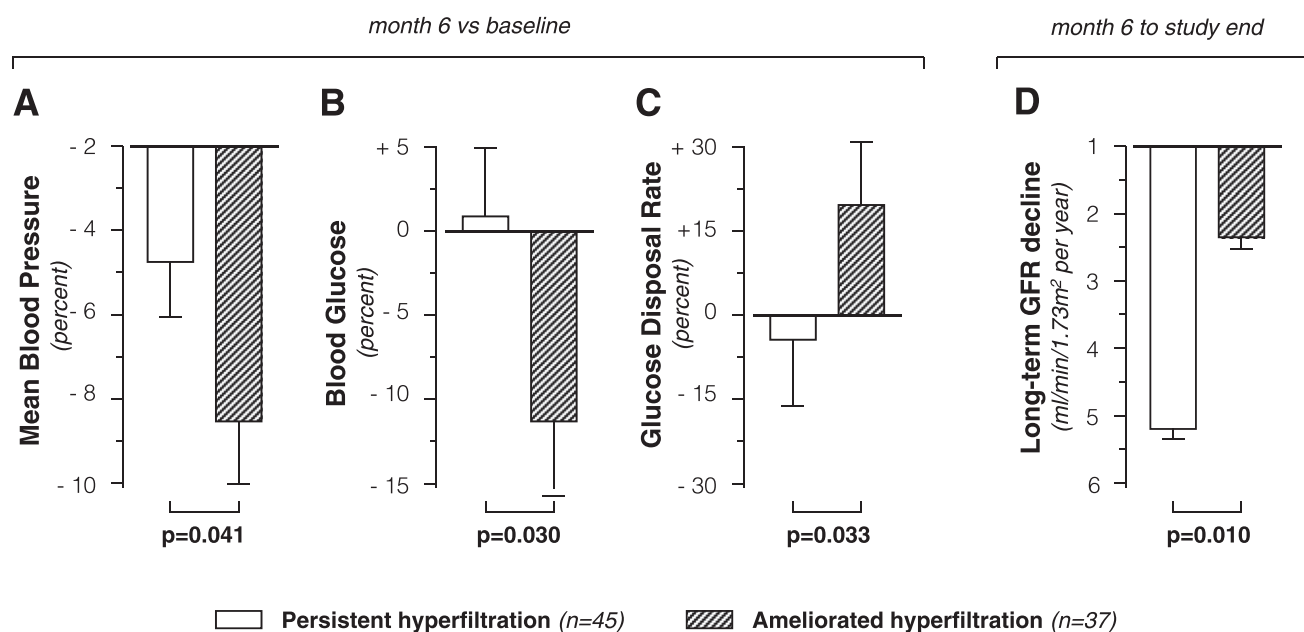


Figure 1—Percent changes at month 6 vs. baseline in mean arterial pressure (A), blood glucose levels (B), and GDR (C) and subsequent GFR decline from month 6 to study end (D) in patients with persistent hyperfiltration compared with patients who had their hyperfiltration at inclusion ameliorated at 6 months. Data are mean and SE.

independent of concomitant changes in HbA_{1c} and BP (Supplementary Table 4, model 2). Albuminuria, randomization to ACE inhibitor therapy, and treatment arm were not significantly associated with any considered outcome. Consistent with results from the BENEDICT and DEMAND trials (10,11), the likelihood ratio tests that evaluated the possibility for considering treatments as a random effect were not significant in model 1 (χ^2 [df1] = 1.10, P = 0.57) or in both versions of model 2 (χ^2 [df1] = 0.43, P = 0.51; and χ^2 [df1] = 1.54, P = 0.46). Thus, treatments were considered only as a fixed effect (Supplementary Table 4). The bimodal GFR change over time was confirmed by sensitivity analyses that through application of a spline function to GFR measurements with a knot at 6 months, showed a significant change of GFR slope across the knot with a negative slope from baseline to month 6 and a positive slope from month 6 to study end (coefficient [SE]: -0.63 [0.72], P < 0.0001 vs. 0.38 [0.77], P < 0.0001, respectively).

UAE. Of the 600 included subjects, 549 (91.5%) had at least one follow-up measurement of albuminuria. Of these subjects, 62 (11.3%) developed micro- or macroalbuminuria. The event was observed in 11 of the 47 patients (23.4%) with persistent hyperfiltration as compared with 53 of the 502 patients (10.6%, P = 0.011) who were already normofiltering at inclusion or no longer hyperfiltering at month 6 (log rank: 6.13, P = 0.013) (Fig. 2). On follow-up, changes in GDR were significantly different between groups (ANCOVA, P = 0.038), and at one year, the GDR was significantly lower in persistently hyperfiltering subjects as compared with all other subjects considered as a whole (3.85 ± 2.17 vs. 5.31 ± 2.45 mg/kg/min, P = 0.024). The above findings were not explained by the distribution of antihypertensive medications, ACE inhibitors, and antidiabetic treatments, which was similar between groups (Supplementary Table 1). Similar findings were observed at sensitivity analyses considering progression to micro- or

macroalbuminuria in patients with persistent hyperfiltration as compared with those who had their hyperfiltration at inclusion ameliorated at month 6 (data not shown).

At multivariable Cox regression, the risk of micro- or macroalbuminuria was not associated with GFR (hazard ratio [HR] 1.0, P = 0.97) or hyperfiltration (1.6, P = 0.10) at baseline, but it was significantly associated with persistent hyperfiltration at month 6 (Fig. 2). The association also was significant after adjusting for predefined baseline covariates, including albuminuria, randomization to ACE inhibition, treatment arm, or inclusion in the BENEDICT-B or DEMAND trial (Fig. 2).

Sensitivity analyses

Long-term GFR decline from baseline to study end, short-term GFR changes from baseline to month 6, and subsequent GFR decline from month 6 to study end were similar when higher GFR cutoff values (125 mL/min/ 1.73 m²) or GFR values not adjusted for BMI were used to define hyperfiltration. Short-term GFR changes and long-term GFR decline were similar when cohorts of patients with at least three, four, or five GFR measurements available for the analyses were considered.

CONCLUSIONS—Serial GFR measurements with gold standard technique in a large cohort of Caucasians with type 2 diabetes allowed us to show that glomerular filtration progressively declines in this population, even before the onset of overt renal disease. Despite intensified therapy, long-term GFR decline averaged 3 mL/min/ 1.73 m² per year, a decline three- to five-fold faster than that reported in the general population (24,25). We also found that 1) 15% of subjects were hyperfiltering at inclusion; 2) more GFR reduction at 6 months predicted slower GFR decline on follow-up, independently of concomitant therapy with ACE inhibitors and level of initial albuminuria; 3) long-term GFR decline and progression to micro- or macroalbuminuria were faster in subjects with persistent hyperfiltration as compared with nonhyperfiltering subjects and with those who had their hyperfiltration at inclusion ameliorated by intensified BP and metabolic control on follow-up; and 4) the above differences in disease outcomes were associated with less effective BP and metabolic control in those with persistent hyperfiltration, despite similar treatment in both groups.

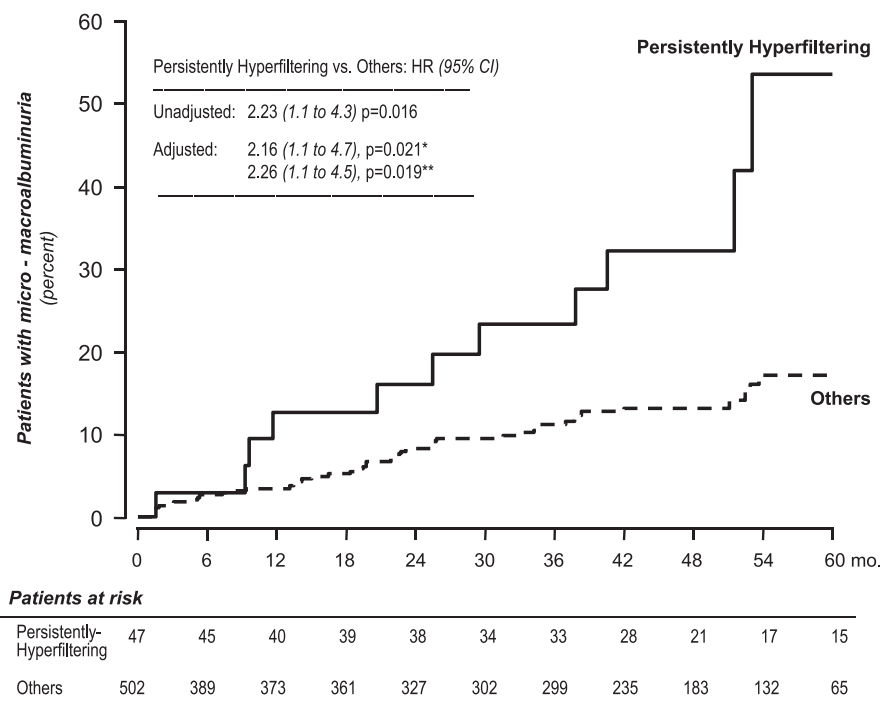


Figure 2—Progression to micro- or macroalbuminuria. Kaplan-Meier survival analysis of patients with persistent hyperfiltration at month 6 (persistently hyperfiltering) compared with all other patients who were already normofiltering at inclusion or were hyperfiltering at inclusion and had their hyperfiltration ameliorated at month 6 (others) (log rank: 6.13, P = 0.013). Unadjusted and adjusted HRs are shown in the accompanying table. *Adjustment for albuminuria at baseline. **Adjustments for age, sex, and albuminuria; HbA_{1c} and systolic BP at baseline; smoking habit; known duration of diabetes; participation in the BENEDICT or DEMAND trial; treatment arm; and treatment with an ACE inhibitor yes or no. Mo, month.

Our data finding that in most subjects the GFR at inclusion was within the normal range, was in harmony with data from other remarkably smaller series of Caucasians with type 2 diabetes (5,26) and may reflect the good metabolic and BP control in this population. However, patients showed a remarkably fast GFR decline in the long-term that was independent of the extent of albuminuria (normo- vs. microalbuminuria) and of concomitant treatment with or without ACE inhibitors. These findings extend to patients with normoalbuminuria previous evidence of accelerated renal function loss in type 2 diabetic patients with microalbuminuria (6) and converge to indicate that in this population, hyperglycemia, hypertension, and, conceivably, other abnormalities associated with the diabetes milieu may sustain renal disease progression independent of proteinuria (27–29). Indeed, microalbuminuria is a well-established risk factor for overt nephropathy and cardiovascular events but, unlike overt proteinuria (30), has never been associated with accelerated GFR decline in this population. These data also serve to emphasize that early optimized treatment of known risk factors, such as hyperglycemia and hypertension, is likely important to limit renal function loss even before the onset of overt nephropathy. In this regard, ACE inhibitors do not appear to affect the rate of GFR decline in this population (11) but may serve to prevent progression to more advanced stages of renal disease (10,31,32) and to achieve regression from micro- to normoalbuminuria, an effect that is associated with reduced cardiovascular morbidity and mortality (11).

Finding that larger short-term GFR reduction was associated with slower GFR decline on subsequent follow-up was also consistent with similar evidence in patients with proteinuric chronic nephropathies (17) or type 1 diabetes (20). It is conceivable that short-term changes in GFR reflected amelioration of glomerular hemodynamics associated with intensified metabolic and BP control, and slowed GFR decline on follow-up reflected the beneficial effect of this hemodynamic response on long-term progression of diabetic kidney disease (33,34). Finding that subjects who had their hyperfiltration ameliorated during the study had a rate of progression to micro- or macroalbuminuria similar to that observed in nonhyperfiltering subjects provided additional evidence that hyperfiltration may have

a role in the pathogenesis of diabetic renal disease and that its amelioration may be renoprotective (1,20,35). Of note, in our series, persistently hyperfiltering subjects, compared with those who had their hyperfiltration ameliorated at month 6, had less effective metabolic and BP control despite similar treatment. Finding that these patients also had lower GDR at inclusion and worsening GDR on follow-up suggests that they were to some extent less responsive to treatment and predisposed to excess risk of renal disease because of more severe insulin resistance (36). Addressing whether this was explained by intrinsic patient characteristics or acquired/environmental factors was beyond the purposes of the current study. On the other hand, our present data show that intensified treatment of known risk factors is not sufficient to halt renal function loss in most patients with type 2 diabetes. Indeed, a substantial proportion of our patients showed persistent hyperfiltration despite optimized metabolic and BP control. This suggests that mechanisms not appreciably affected by available treatments, such as oxidative stress, endothelial dysfunction, ischemia, accelerated ageing, and probably others, may play a role in progressive kidney dysfunction in this population, even before onset of overt nephropathy (30).

Defining hyperfiltration as a GFR exceeding the upper limit of the normal range of the iothexol plasma clearance technique was to some extent arbitrary but, as in previous studies with a similar approach (37), was aimed to identify a priori a pure population of hyperfiltering subjects for comparative analyses versus subjects with lower GFRs at inclusion. On the other hand, we could not exclude the presence of subjects with relative hyperfiltration within the cohort with baseline GFR <120 mL/min/1.73 m². Finding that the outcomes of the two groups were significantly different, despite the possible dilution effect of these subjects, provided additional evidence of the pathogenic role of glomerular hyperfiltration in this setting. On the other hand, finding that higher GFR also was an independent predictor of accelerated GFR decline when the GFR was considered as a continuous variable, provided the additional information that hyperfiltration was a risk factor throughout the whole range of considered GFR values at inclusion. Thus, measuring the GFR, in addition to albuminuria, may help in

identifying patients at increased risk because of persistent hyperfiltration despite optimized metabolic and BP control and who might benefit the most from early intervention with novel treatments targeting potential mediators of accelerated renal dysfunction in addition to hyperglycemia and arterial hypertension (30).

Limitations and strengths

A weakness of the study is that this was a post hoc observational analysis of subjects included in trials originally designed for other purposes. Thus, study findings are hypothesis generating and need to be tested in ad hoc prospective studies. By design, our study could not examine hard end points, such as kidney failure or doubling of serum creatinine levels. On the other hand, inclusion of subjects without evidence of overt nephropathy allowed us to evaluate the pathogenic role of renal functional abnormalities in early stages of diabetic renal disease. The number of persistently hyperfiltering patients was relatively small. Our present data, however, reflected the relatively small prevalence of persistent hyperfiltration in our population and provided novel information that allows us to weigh up the actual role of hyperfiltration in type 2 diabetes. The major strengths were that our study was remarkably larger and longer than any previous study serially evaluating GFR decline in diabetic populations and that all subjects were prospectively monitored by gold standard procedures. The results may have a large external validity since our study population had clinical characteristics, such as hypertension and normo- or microalbuminuria, that are common to the large majority of type 2 diabetic subjects. Moreover, the fact that GFR data were available for all patients from DEMAND and the vast majority of those from BENEDICT allows generalization of the results of slope analyses to the average population of type 2 diabetic patients with normo- or microalbuminuria. Data reliability was confirmed by sensitivity analyses showing that study results were independent of the criteria used for the definition of glomerular hyperfiltration and the number of GFR measurements required for patient inclusion in statistical analyses.

Conclusion

Results of our observational post hoc analyses suggest that in hypertensive type 2 diabetic subjects with normo- or microalbuminuria, persistent hyperfiltration is

an independent risk factor for accelerated renal function loss and development or progression of nephropathy, whereas amelioration of hyperfiltration is renoprotective. Prospective ad hoc studies are needed to unravel the mechanisms underlying persistent hyperfiltration despite optimized metabolic and BP control and to assess whether and to what extent glomerular hyperfiltration can be a specific treatment target for novel interventions aimed to limit renal function loss in this population.

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P.R. and G.R. conceived the study, contributed to data analyses and interpretation, and wrote the manuscript. E.L.P. conceived the study, performed the statistical analyses, contributed to data analyses and interpretation, and wrote the manuscript. F.G. coordinated the activities of the laboratory of the Clinical Research Center where all the GFR measurements were centralized. N.M. performed the statistical analyses. A.C., F.C., C.C., S.F., and N.S. performed all the laboratory analyses. A.P., I.I., A.R.D., R.T., A.B., and J.Z. contributed to patient identification, monitoring, and care. All authors had full access to and take full responsibility for the integrity of data and critically revised and approved the manuscript. P.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM. Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. *J Clin Invest* 1986;77:1925–1930
2. Magee GM, Bilous RW, Cardwell CR, Hunter SJ, Kee F, Fogarty DG. Is hyperfiltration associated with the future risk of developing diabetic nephropathy? A meta-analysis. *Diabetologia* 2009;52:691–697
3. Yip JW, Jones SL, Wiseman MJ, Hill C, Viberti G. Glomerular hyperfiltration in the prediction of nephropathy in IDDM: a 10-year follow-up study. *Diabetes* 1996;45:1729–1733
4. Silveiro SP, Friedman R, de Azevedo MJ, Canani LH, Gross JL. Five-year prospective study of glomerular filtration rate and albumin excretion rate in normofiltering and hyperfiltering normoalbuminuric NIDDM patients. *Diabetes Care* 1996;19:171–174
5. Fontseré N, Salinas I, Bonal J, et al. Are prediction equations for glomerular filtration rate useful for the long-term monitoring of type 2 diabetic patients? *Nephrol Dial Transplant* 2006;21:2152–2158
6. Andersen S, Bröchner-Mortensen J, Parving HH; Irbesartan in Patients With Type 2 Diabetes and Microalbuminuria Study Group. Kidney function during and after withdrawal of long-term irbesartan treatment in patients with type 2 diabetes and microalbuminuria. *Diabetes Care* 2003;26:3296–3302
7. Moriya T, Tsuchiya A, Okizaki S, Hayashi A, Tanaka K, Shichiri M. Glomerular hyperfiltration and increased glomerular filtration surface are associated with renal function decline in normo- and microalbuminuric type 2 diabetes. *Kidney Int* 2012;81:486–493
8. Chaiken RL, Eckert-Norton M, Bard M, et al. Hyperfiltration in African-American patients with type 2 diabetes. Cross-sectional and longitudinal data. *Diabetes Care* 1998;21:2129–2134
9. Nelson RG, Bennett PH, Beck GJ, et al.; Diabetic Renal Disease Study Group. Development and progression of renal disease in Pima Indians with non-insulin-dependent diabetes mellitus. *N Engl J Med* 1996;335:1636–1642
10. Ruggenenti P, Fassi A, Ilieva AP, et al.; BENEDICT-B Study Investigators. Effects of verapamil added-on trandolapril therapy in hypertensive type 2 diabetes patients with microalbuminuria: the BENEDICT-B randomized trial. *J Hypertens* 2011;29:207–216
11. Ruggenenti P, Lauria G, Iliev IP, et al.; DEMAND Study Investigators. Effects of manidipine and delapril in hypertensive patients with type 2 diabetes mellitus: the Delapril and Manidipine for Nephroprotection in Diabetes (DEMAND) randomized clinical trial. *Hypertension* 2011;58:776–783
12. Gaspari F, Perico N, Ruggenenti P, et al. Plasma clearance of nonradioactive io-hexol as a measure of glomerular filtration rate. *J Am Soc Nephrol* 1995;6:257–263
13. Gaspari F, Perico N, Matalone M, et al. Precision of plasma clearance of io-hexol for estimation of GFR in patients with renal disease. *J Am Soc Nephrol* 1998;9:310–313
14. Ruggenenti P, Perna A, Gherardi G, Gaspari F, Benini R, Remuzzi G. Renal function and requirement for dialysis in chronic nephropathy patients on long-term ramipril: REIN follow-up trial. Gruppo Italiano di Studi Epidemiologici in Nefrologia (GISEN). Ramipril Efficacy in Nephropathy. *Lancet* 1998;352:1252–1256
15. Gaspari F, Ferrari S, Stucchi N, et al.; MY.S.S. Study Investigators. Performance of different prediction equations for estimating renal function in kidney transplantation. *Am J Transplant* 2004;4:1826–1835
16. Parvanova AI, Trevisan R, Iliev IP, et al. Insulin resistance and microalbuminuria: a cross-sectional, case-control study of 158 patients with type 2 diabetes and different degrees of urinary albumin excretion. *Diabetes* 2006;55:1456–1462
17. Beck GJ, Berg RL, Coggins CH, et al.; The Modification of Diet in Renal Disease Study Group. Design and statistical issues of the Modification of Diet in Renal Disease Trial. *Control Clin Trials* 1991;12:566–586
18. Gassman JJ, Greene T, Wright JT Jr, et al. Design and statistical aspects of the African American Study of Kidney Disease and Hypertension (AASK). *J Am Soc Nephrol* 2003;14(Suppl. 2):S154–S165
19. BENEDICT Group. The BERGamo NEphrologic Diabetes Complications Trial (BENEDICT): design and baseline characteristics. *Control Clin Trials* 2003;24:442–461
20. Hansen HP, Rossing P, Tarnow L, Nielsen FS, Jensen BR, Parving HH. Increased glomerular filtration rate after withdrawal of long-term antihypertensive treatment in diabetic nephropathy. *Kidney Int* 1995;47:1726–1731
21. Barnett AG, van der Pols JC, Dobson AJ. Regression to the mean: what it is and how to deal with it. *Int J Epidemiol* 2005;34:215–220
22. Higgins JP, Whitehead A, Turner RM, Omar RZ, Thompson SG. Meta-analysis of continuous outcome data from individual patients. *Stat Med* 2001;20:2219–2241
23. Cnaan A, Laird NM, Slasor P. Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. *Stat Med* 1997;16:2349–2380
24. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in

- renal function with age. *J Am Geriatr Soc* 1985;33:278–285
25. Rowe JW, Andres R, Tobin JD, Norris AH, Shock NW. The effect of age on creatinine clearance in men: a cross-sectional and longitudinal study. *J Gerontol* 1976;31:155–163
26. Chudleigh RA, Dunseath G, Evans W, et al. How reliable is estimation of glomerular filtration rate at diagnosis of type 2 diabetes? *Diabetes Care* 2007;30:300–305
27. Bash LD, Selvin E, Steffes M, Coresh J, Astor BC. Poor glycemic control in diabetes and the risk of incident chronic kidney disease even in the absence of albuminuria and retinopathy: Atherosclerosis Risk in Communities (ARIC) Study. *Arch Intern Med* 2008;168:2440–2447
28. Kramer HJ, Nguyen QD, Curhan G, Hsu CY. Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. *JAMA* 2003;289:3273–3277
29. Pavkov ME, Knowler WC, Lemley KV, Mason CC, Myers BD, Nelson RG. Early renal function decline in type 2 diabetes. *Clin J Am Soc Nephrol* 2012;7:78–84
30. Ruggenti P, Remuzzi G. Time to abandon microalbuminuria? *Kidney Int* 2006;70:1214–1222
31. Parving HH, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Arner P; Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study Group. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 2001;345:870–878
32. Ruggenti P, Fassi A, Ilieva AP, et al.; Bergamo Nephrologic Diabetes Complications Trial (BENEDICT) Investigators. Preventing microalbuminuria in type 2 diabetes. *N Engl J Med* 2004;351:1941–1951
33. Parving HH, Andersen AR, Smidt UM, Svendsen PA. Early aggressive antihypertensive treatment reduces rate of decline in kidney function in diabetic nephropathy. *Lancet* 1983;1:1175–1179
34. Thomaseth K, Pacini G, Morelli P, Tonolo G, Nosadini R. Importance of glycemic control on the course of glomerular filtration rate in type 2 diabetes with hypertension and microalbuminuria under tight blood pressure control. *Nutr Metab Cardiovasc Dis* 2008;18:632–638
35. Wright JT Jr, Bakris G, Greene T, et al.; African American Study of Kidney Disease and Hypertension Study Group. Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. *JAMA* 2002;288:2421–2431
36. Velloso LA, Folli F, Perego L, Saad MJ. The multi-faceted cross-talk between the insulin and angiotensin II signaling systems. *Diabetes Metab Res Rev* 2006;22:98–107
37. Jerums G, Premaratne E, Panagiotopoulos S, MacIsaac RJ. The clinical significance of hyperfiltration in diabetes. *Diabetologia* 2010;53:2093–2104